The complete amino acid sequence of the bacteriochlorophyll *c* binding polypeptide from chlorosomes of the green photosynthetic bacterium *Chloroflexus aurantiacus*

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The BChl*c* polypeptide was isolated from chlorosomes of the green bacterium *Chloroflexus aurantiacus* on Sephadex LH-60. The complete amino acid sequence of this 5.6 kDa polypeptide (51 amino acid residues) was determined. Most probably the 5.6 kDa polypeptide forms an α-helix between Trp 5 and Ile 42 with an asymmetrical (bipolar) distribution of polar amino acid residues along the helix axis: (i) At one side of the α-helix 5 Gln and 2 Asn residues are the possible binding sites for 7 BChl*c* molecules. (ii) On the other side Ser, Thr, His residues seem to be polypeptide-polypeptide interaction sites within the BChl*c*-protein complexes. It appears that the BChl-protein complex (chlorosome subunit, 5.2 × 6 nm) composed of 12 5.6 kDa polypeptides corresponds to the 'globular units' found by electron microscopy within the chlorosomes.

Chloroflexus aurantiacus *Chlorosome* *Light-harvesting polypeptide* *BChlc binding polypeptide* *Amino acid sequence*

1. INTRODUCTION

The photosynthetic apparatus of the green thermophilic bacterium *Chloroflexus aurantiacus* contains two types of light-harvesting complexes: (i) the B 808-865 complex binding BChl*a* and located in the cytoplasmatic membrane, and (ii) as primary light absorber the B 740 complex binding BChl*c* and specifically organized in chlorosomes (100 × 30 nm) [1–4]. The supramolecular architecture of the chlorosomes and the associated cytoplasmatic membranes were investigated by freeze-fracture electron microscopy [5] and the antenna organization and the function of antenna pigments (BChl*a*, *c*) were studied by spectroscopic methods [6]. Recently, the isolation and identification of BChl*a* and BChl*c* binding polypeptides (incl. one reaction center polypeptide) together with their localization in the cytoplasmatic membrane or the chlorosomes, respectively, was described [7]. Three major polypeptides (M_r 18000, 11000 and 3700) and a fourth polypeptide (M_r 5800) in minor quantities were isolated from purified chlorosomes. The 3.7 kDa polypeptide was postulated to bind antenna BChl*c*.

A thorough primary structure analysis of the BChl*a* antenna polypeptides from purple photosynthetic bacteria [8–12] has been carried

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Abbreviations: BChl, bacteriochlorophyll; PTH, phenylthiohydantoin; CIM/NH_4OAc/HOAc, chloroform/methanol (1 : 1, v/v) containing 0.1 M ammonium acetate and 20% acetic acid; PAGE, polyacrylamide gel electrophoresis

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out. In addition, a water-soluble BChl*a* protein complex from the green bacterium *Prothecochloris aestuarii* has been isolated and its 3-dimensional structure determined [13]. However, no information is available on the major light-harvesting BChl*c*-protein complex of the chlorosomes in either of the families (Chlorobiaceae or Chloroflexiaceae) of the green photosynthetic bacteria. Here, we report on the isolation and amino acid sequence analysis of the bacteriochlorophyll *c* binding polypeptide. Evidence is also presented on the possible structural organization of this polypeptide and its BChl*c* interactions within the chlorosome.

2. MATERIALS AND METHODS

Cells were grown anaerobically at low incident light intensities in 14 l fermenters as previously described [14]. This mode of growth yields large amounts of BChl*c* [14]. The organic solvent soluble polypeptides from *C. aurantiacus* (strain J-10 fl) were extracted from both lyophilized whole cells and chlorosomes with CIM (1:1, v/v), 0.1 M NH_4OAc, 20% HOAc, as in [9]. The chlorosomes were prepared by the procedure reported in [7]. The 5.6 kDa polypeptide was separated from pigments and the other chlorosome polypeptides by gel filtration on Sephadex LH-60 (Pharmacia) on a column: 3 × 150 cm. Fractions containing the 5.6 kDa polypeptide were dialyzed extensively against water and lyophilized. For amino acid analysis polypeptide samples (50–100 nmol) were hydrolyzed in 0.1–0.2 ml of constantly boiling 6 N HCl (110°C) in vacuo. The samples were hydrolyzed for 24, 48, 72 and 160 h. After drying, the samples were analyzed on a Biotronic LC 6000 E analyzer. Tryptophan determination was done after hydrolysis with 6 N methanesulfonic acid containing 0.2% tryptamine (Fluka) [15].

The amino acid sequence of the polypeptide was determined either by automatic Edman degradation in a Beckman 890C sequencer using the Quadrol (0.25 M) program together with Polybrene (Sigma) as an additive [16], or by a slightly modified manual Edman degradation procedure [17]. PTH-amino acid derivatives were identified by the HPLC method as described in [18]. PTH-Arg and PTH-His were identified on an isocratic HPLC system on Pertisil 5 PAC (Whatman) [19]. Identification of the carboxyterminal amino acid residues was performed enzymatically with carboxypeptidase B and A (Boehringer) at pH 8.5 [21]. Aliquots of the carboxypeptidase digest were removed after 0, 0.5, 1.5, 9, 120 and 180 min, boiled, lyophilized and subjected to amino acid analysis.

3. RESULTS

The starting material for the BChl*c* binding polypeptide isolation was mainly purified chlorosomes containing only four polypeptides as previously described [4,14]. The polypeptide was isolated in a one-step procedure by gel filtration on Sephadex LH 60 after dissolving the chlorosomes in CIM/NH_4OAc/20% HOAc. The elution diagram is shown in fig.1. Fractions of the peaks I, II and III contained the 18 kDa and 11 kDa polypeptides, respectively [7], whereas the pure BChl*c* binding polypeptide was found in peak IV. SDS-PAGE of fraction peak IV showed one single band with an apparent M_r of 4000. The BChl*c* binding polypeptide was previously determined to have an M_r 3700–5500 on SDS-PAGE [7]. Now by sequence analysis (see below) it has a true M_r of 5592 and is renamed 5.6 kDa polypeptide. After dialysis and lyophilization this polypeptide material of fraction IV was used for amino acid

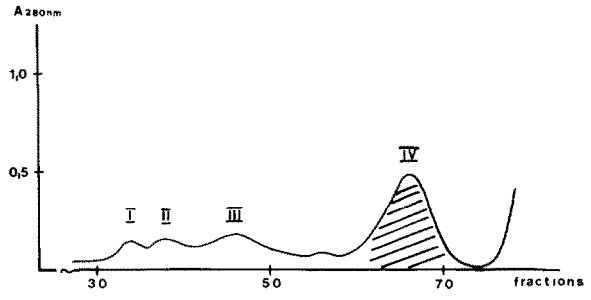

Fig.1. Gel filtration of the organic solvent soluble polypeptide extract from chlorosomes (45 mg) on Sephadex LH-60 in CIM 1:1/0.1 M NH_4OAc/20% HOAc; 6 ml/fraction. I, 18 kDa polypeptide and small amounts of the 11 kDa polypeptide. II, mixture of the 18 kDa and 11 kDa polypeptides. III, 11 kDa polypeptide and small amounts of the 18 kDa polypeptide. IV, 5.6 kDa polypeptide.

Table 1

Amino acid composition of the 5.6 kDa polypeptide

Amino acid	Number of residues[a]			Amino acid	Number of residues[a]		
	A[b]	B	C		A	B	C
Asx	6.1	6	6	Met	0.6	1	1
Thr	1.9	2	2	Ile	3.0	3	3
Ser	4.2	4	4	Leu	1.1	1	1
Glx	5.8	6	6	Tyr	1.1	1	1
Pro	0.8	1	1	Phe	2.2	2	2
Gly	4.2	4	4	His[a]	1.0	1	1
Ala	9.2	9	9	Arg	3.5	3	3
Val	4.0	4	4	Trp[c]	3.2	3	3

[a] Data are based on 1 His residue per polypeptide chain
[b] Results are expressed in mol residue/mol polypeptide
[c] Methanesulfonic acid hydrolysis (24 h) according to [15]

(A) 48-h hydrolysis; (B) nearest integer; (C) number of residues as derived from sequence

analysis and sequence analysis without further purification.

The amino acid composition of the 5.6 kDa polypeptide after the LH-60 purification step is shown in table 1. The data are based on one His residue per one polypeptide molecule. The number of amino acid residues obtained after 24, 48, 72 and 160 h hydrolysis agrees well with those from amino acid sequence analysis (in table 1 only the 48 h values are shown). No lysine and cysteine and only one Met and His residue were found. The high amount of both the hydrophobic residues Ala, Val, Tyr, Phe, Trp (adding up about to 1/2 of the whole molecule) and of Asx and Glx (12 residues) is of particular interest for structural reasons (see below). The polarity is 43%.

Two samples of the 5.6 kDa polypeptide were subjected to manual Edman degradation. Before this procedure, one of these samples had been treated with chloroform/methanol/5% HCl to deformylate a possible blocked N-terminus (*N*-formyl-methionine) [8]. In both cases Ala was released as the only N-terminal amino acid. Enzymatic digestion with carboxypeptidase B released Arg, while treatment with carboxypeptidase B followed by carboxypeptidase A digestion produced Arg and Val. Glycine as a third amino acid was very slowly released. From these enzymatic degradation experiments the C-terminal amino acid sequence -Gly-Val-Arg-COOH was deduced (fig.2). Amino acid sequence analysis by automated Edman degradation liberated in one run 50 of the 51 amino acid residues. The C-terminal Arg 51 was not released. The results of the Edman degradation are shown in fig.2. The only His residue (position 24) is located within a cluster of the aromatic residues (Phe 21 and Trp 25, 27) in the middle of the polypeptide chain. An interesting feature is the high amount of Asn and Gln residues compared to the number of free acids. Based on the amino acid sequence data, this polypeptide has an M_r of 5592.

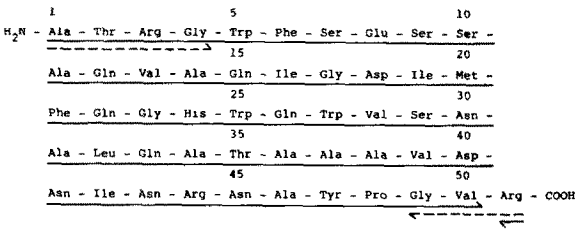

Fig.2. Amino acid sequence of the 5.6 kDa polypeptide from chlorosmes of *C. aurantiacus* J-10-fl. —→, Automatic sequence determination (Edman degradation) in the sequencer. - - - →, By the manual Edman procedure. ←—, Digestion with carboxypeptidase B. ←- - -, Digestion with carboxypeptidase B and A.

4. DISCUSSION

Previous degradation experiments of the three polypeptides of the chlorosome from *C. aurantiacus* by trypsin or chymotrypsin and subsequent changes in intensity of the absorption band at 740 nm indicated the binding of all of the BChl*c* with the small 5.6 kDa (3.7–5.5 kDa) polypeptide [7]. The size of the 5.6 kDa polypeptide with 51 amino acid residues is similar to the size of the light-harvesting BChl*a* polypeptides of purple photosynthetic bacteria [8–12]. However, the stoichiometric relationship between BChl and the polypeptide is significantly different. On the basis of the analytical data with isolated chlorosomes and the molecular mass of 5.6 kDa, it can be assumed that up to 8 BChl*c* molecules are bound per polypeptide chain [7]. A similar high number of BChl*c* molecules (12–14) per in vivo aggregate (protein subunit) of the antenna rod elements of Chlorobiaceae was found by Smith et al. [22] (ESR line width) and Olson [23], if one takes into account the formation of dimers of the 5.6 kDa polypeptide (as the protein subunit) with 16 BChl per dimer. In fact, dimer formation of this small BChl*c* polypeptide was demonstrated by crosslinking experiments of purified membranes [7].

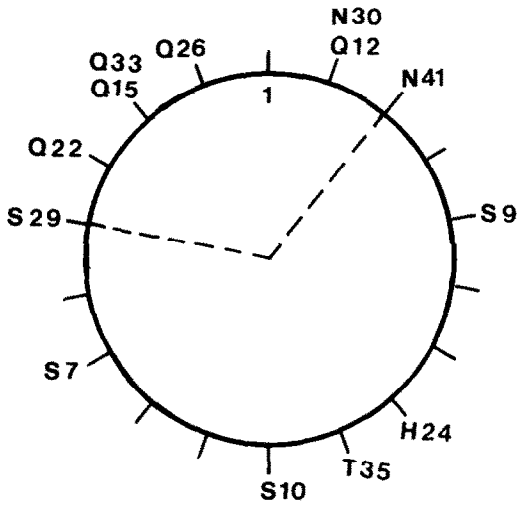

Fig.3. Hypothetical α-helix [24] of the 5.6 kDa polypeptide from the chlorosomes of *C. aurantiacus* between Trp 5 and Ile 42. α-Helical wheel projection according to [25]. The view is a projection down the helical axis with successive C_α-positions rotated along the projected peptide backbone (origin (position 1) of the numbering at the N-terminus). The direction of the projected side chains are indicated by spokes emanating from the wheel center. Only the polar residues Gln, Asn, Ser, Thr and His are shown.

Secondary structure predictions on the basis of the primary structure data [24] of the 5.6 kDa polypeptide (23 non-polar and 23 polar plus charged residues) indicated preferentially α-helix formation between Trp 5 and Ile 42 (38 residues, helix length 57 Å). Most interestingly (and supporting the α-helical structure) all 5 Gln and 2 Asn residues found in the α-helix are located at one side of this α-helix, whereas the Ser, Thr residues plus His (altogether 4) are located on the other side of the α-helix (α-helical wheel, fig.3 [25]). The three residual polar residues are in the non-helical part of the polypeptide chain. The distinct sidedness of the α-helix is probably related to the binding of the BChl*c* molecules or the interaction between the polypeptides: (i) The 7 Gln/Asn residues are possible interaction sites with the 7 BChl*c* molecules [26]. (ii) Via the Ser, Thr residues on the other side of the α-helix and by polar residues outside the α-helix, the polypeptide chains could interact to form higher aggregates, i.e., the BChl*c*-protein complexes [9,11]. On the basis of a Nicholson molecular model it can be postulated that 7 BChl*c* are bound via the central Mg atom to Gln and Asn residues (group I: Gln 12, 15, 22 and Group II: Gln 26, 30, Asn 33, 41). They could further interact with one another by hydrogen-bonded nucleophilic interactions between the OH-group of ring I and the C = O group of ring V (fig.4A). In the center of the α-helix there is a cluster of aromatic residues (Phe 21, Trp 25, 27, for the functional importance of these residues, see [9]). The 5.6 kDa polypeptide contains no lysine residues which could be crosslinked by an amino group specific reagent like dimethyl 3,3′-dithiobis(propionimidate). Therefore, crosslinking with this reagent to form dimers [7] can only occur by the N-terminal α-amino groups of the 5.6 kDa polypeptide. The high yield of the crosslinking reactions (up to 90%) indicated either a dimeric basic unit or tight packing of an equal number of α-helices with equal distances in vivo within the light-harvesting complex.

On the basis of this dimeric unit and the asymmetry of the α-helix with respect to the polar residues (Gln/Asn: BChl*c* binding; Ser/Thr: polypeptide-polypeptide interactions) one can sug-

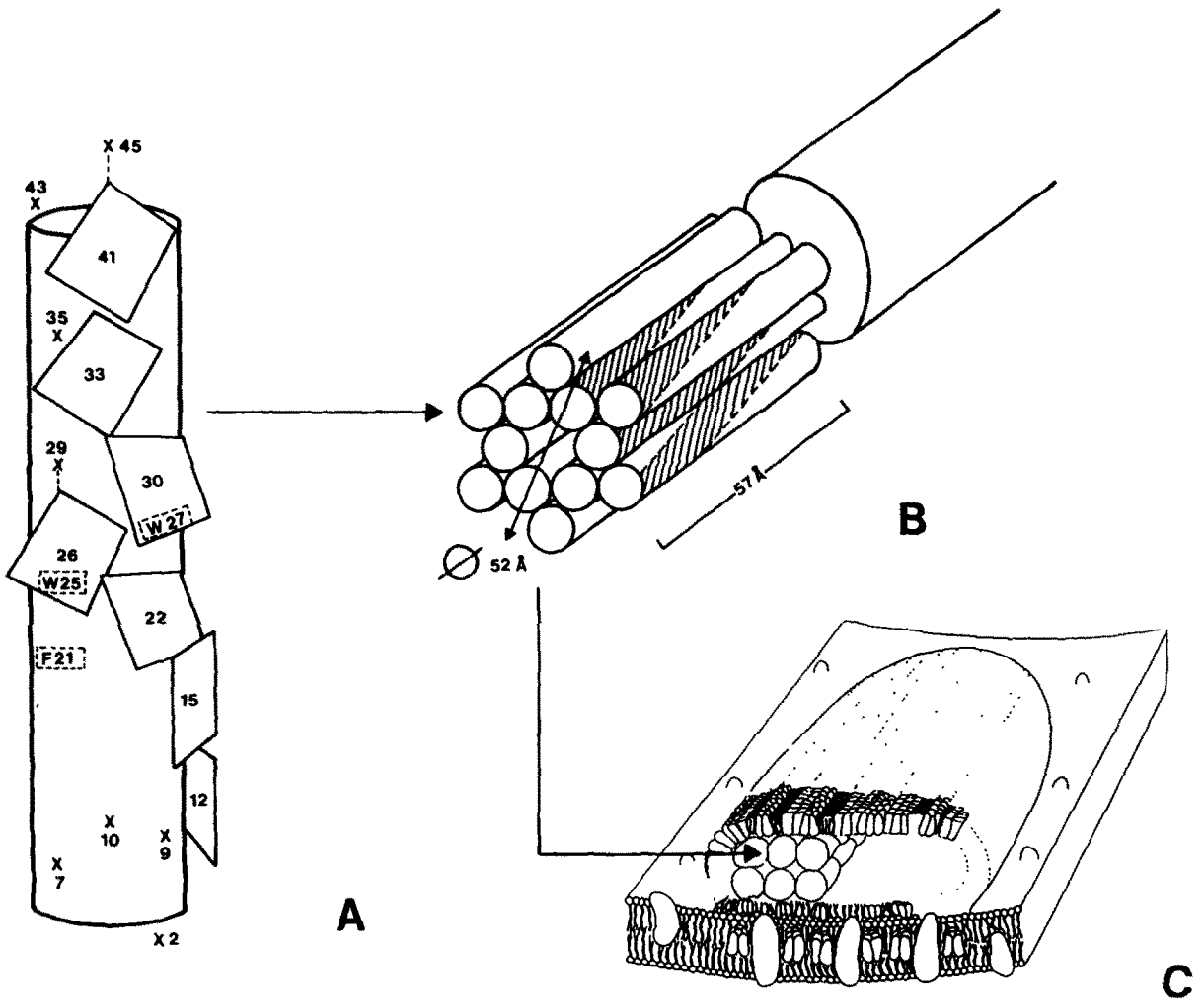

Fig.4. (A) Proposed model of the 5.6 kDa polypeptide α-helix with possible BChlc binding sites (Gln 12, 15, 22, 26, 33; Asn 30, 41) via the central Mg atom. The BChlc molecules interact with one another by hydrogen-bonded nucleophilic interactions (C=O group of ring V and -CH(CH_3)-OH-group of ring I) or to Ser 29 and Asn 45, respectively. Residues X 2, X 7, X 9, X 10, X 35, X 43 in the back of the α-helix are Ser, Thr residues involved in polypeptide-polypeptide interactions; F21, W25, W27 form the aromatic cluster region in the center of the α-helix. (B) Chlorosome subunit ('globular subunit'), light-harvesting BChlc-protein complex of *C. aurantiacus* composed of 12 polypeptide chains (α-helices) of the 5.6 kDa polypeptide. Pairs of α-helices (dimeric basic units) are tilted to expose the areas for BChlc binding (hatched areas). (C) Chlorosome model (cross-section, *C. aurantiacus*) containing the chlorosome subunits ('globular subunits') of the rod-shaped elements in the chlorosome core region [5,7].

gest a chlorosome subunit (BChlc-protein complex) composed of 6 dimers (12 polypeptide chains) and 84 BChlc molecules located on the surface of such a unit (fig.4B). The dimensions of this unit correspond to those of the 'globular units' of the rod-shaped elements in the chlorosome [5]: 5.2 nm in diameter and 6 nm in length (length of the α-helix, fig.4B,C). This agreement in size would strongly support the α-helical conformation of the 5.6 kDa polypeptide. On the surface of the α-helix (α-helix pair) or the chlorosome subunit, the BChlc molecules could be organized in an oligomeric arrangement having their transition dipoles oriented about parallel (angle nearly 40° [6]) to the axis of

the α-helix or the long axis of the chlorosome subunits. A similar organization was postulated within the whole chlorosome on the basis of spectroscopic data [6,22].

The primary structure of the antenna BChlc polypeptide from the chlorosomes of *C. aurantiacus* and the pigment-polypeptide association proposed (fig.4) confirms and extends the proposals put forward previously for the ultrastructural organization of the chlorosome based on electron microscopic, spectral and biochemical analyses [5–7]. It also represents a new type of light-harvesting pigment-protein complex.

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